[0264] 7. The samples were brought to dry ethanol over two days;

[0265] 8. The samples were vacuum dried at 40° C. and transferred to pure dry hexamethylene di-isocyanate at 80° C. under dry nitrogen for two days;

[0266] 9. Excess hexamethylene di-isocyanate was removed from the material as follows:

[0267] a) Four rinses with cold anhydrous acetone followed by refluxing in anhydrous acetone overnight at 60° C.;

[0268] b) Water added to hydrolyse any remaining CNO groups;

[0269] c) Material dried in an oven initially at 40° C. and finally at 60° C. before autoclaving (no trace of acetone could be detected by smell);

[0270] d) checked for the absence of the CNO peak in the material using fourier transform infra red (FT-IR) spectroscopy.

Example 3

Protocol for Testing Fibroin-Apatite Materials

Mineralisation

[0271] Mineral loadings were determined gravimetrically by heating of the material to 500° C. in air. The preferred embodiment gave loadings of 30% w/w mineral content while modification of the protocol, as described above, gave mineral loadings up to 70% w/w mineral content.

[0272] Samples of the material were further studied by scanning electron microscopy (JEOL JSM 6330) fitted with an energy dispersive X-ray analyser. X-ray energy spectra demonstrated the co-localization of calcium and phosphate within the pore walls (FIGS. **5** and **6**) and the presence of high levels of mineralization with calcium phosphate (FIG. **7**). Evidence of small quantities of chloride ions in the X-ray energy spectra may be accounted for by chlor-substitution of the hydroxyapatite (see below).

[0273] FT-IR spectroscopy (KBr discs; Perkin-Elmer Spectrum 1) confirmed the presence of large quantities of phosphate in the composite (FIG. **8** peaks E and F). Powder X-ray diffraction (Bruker D8) demonstrated the presence of chloride-substituted hydroxyapatite in the composite (FIG. **9**).

Example 4

Protocol for Testing Fibroin-Apatite Materials

Load-Bearing Properties

[0274] Mechanical tests (Zwick 1478) were performed on fully hydrated samples of the material, which were cut into cylinders and compressed with a crosshead speed of 2 mm min^{-1} to destruction.

[0275] The stress/strain curve (FIG. **10**) shows that the material has an extended plastic deformation phase.

[0276] The mean unconfined compressive toughness of the di-isocyanate cross-linked material was 11.93±8.40 kJ m^{-2}, n=6 (obtained using the J-integral method).

[0277] The mean unconfined ultimate compressive strength (stress to yield point) of the material was 14 MPa (n=5).

[0278] The unconfined compressive elastic modulus of the material was 175 MPa (n=5).

[0279] In the case of compressive strength and the compressive elastic modulus, the measured values are reasonably close to the target values for a BRM, being 20 MPa for the compressive strength and 100-500 MPa for the compressive elastic modulus, respectively.

[0280] In the case of toughness, the measured value exceeded target values understood to be advantageous for a BRM, the target value being 1.3 kJ m^{-3}.

Example 5

Protocol for Testing Fibroin-Apatite Materials

Pyrogenicity

[0281] 5 mg samples of the material were inserted into pyrogen-free 1.5 ml polypropylene reaction vials (Eppendorf) with heat-sterilized forceps together with 1000 μl of isotonic saline solution (Berlin-Chemie AG) and either 100 μl of LPS spike (NIBSC, UK; WHO reference, *Escherichia coli,* 0113:H10) diluted in saline, or 100 μl of saline as a control.

[0282] Spiking the samples with LPS (1 or 4 EU/ml) was used to exclude interference from blood monocyte activities, for example from toxic or immuno-modulatory samples. Spike recovery values of between 50-200% were deemed acceptable to exclude interference.

[0283] A standard curve for endotoxin diluted in saline with 0.5 EU/ml as the threshold concentration for pyrogenicity was included in all tests.

[0284] 100 μl of pooled blood obtained from healthy volunteers and checked for infections by differential blood cell counting (Pentra 60, ABX Diagnostics, France) was added to each reaction vial to give a final incubation volume of 1200 μl and left for 21-24 hours at 37° C. and 5% CO^2.

[0285] Cell-free supernatants were obtained by centrifugation at 13,000 rpm for two minutes and assayed immediately, or stored at −80° C. until measurements could be taken.

[0286] Release of IL-1 was detected by ELISA with an antibody pair and recombinant standard (R&D Systems, Wiesbaden, Germany) The detection limit of the ELISA was 3.5 pg/ml IL-1β. The assay demonstrated that the pyrogenicity of the material was negligible (FIG. **11**).

Example 6

Protocol for Testing Fibroin-Apatite Materials

Osteogenicity

[0287] Adult human bone marrow samples were obtained from haematologically normal patients undergoing routine hip replacement surgery for osteoarthritis. Only tissue that would have been discarded was used with the approval of the Southampton and South West Hampshire Local Research Ethics Committee. A total of four samples (two male and two female of mean age 70±13 years) were prepared.

[0288] Primary cultures of bone marrow cells were established, after enrichment by selection for STRO-1 (a marker, from a CD34+ fraction, of pluripotency) using STRO-1 antibody hybridoma supernatant (gift from Dr J Beresford, University of Bath, UK), which facilitates rapid expansion in vitro prior to implantation (S. Gronthos, S. E. Graves, S. Ohta, P. J. Simmons, Blood 84, 4164-4173 (1994)).

[0289] Cultures were maintained in basal medium (MEM with 10% FCS, 1% penicillin/streptomycin) at 37° C. in humidified air with 5% CO^2.